NIH AIDS Research Program Evaluation

AD HOC SUBPANEL ON OPPORTUNISTIC INFECTIONS RESEARCH SUBGROUPS REPORT

Findings and Recommendations

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Introduction

HIV infection results in a gradual, progressive process that damages the immune system of infected individuals and makes them susceptible to a diverse collection of bacteria, viruses, fungi, and protozoa that represent the major causes of suffering and death for HIV-infected people. Indeed, an opportunistic infection (OI) is often the initial sign of the clinical syndrome AIDS. The consequences of OIs can be severe and, indeed, a diverse collection of OIs represents the major causes of suffering and death for HIV-infected people. These pathogens can affect virtually all tissues and organ systems, causing a severe functional compromise and, in some instances, malignant transformation.

Although prophylactic regimens have been defined that decrease substantially the risk of developing certain important OIs, none are completely successful, and all are often complicated by untoward side effects or significant inconvenience. Effective therapeutic strategies have been developed to treat specific OIs once serious infection takes hold; however, none are curative and lifelong. They are often toxic, and suppressive therapy is required following recovery from the acute disease presentation. Cumulatively, these prophylactic and therapeutic advances have made significant contributions to prolonging the lives of people with AIDS, yet there remains a great need to develop more effective and less toxic drugs to prevent and treat OIs for which therapies are now available and to develop effective treatments for a number of OIs for which no effective therapies exist.

Much of the progress made to date in preventing and treating AIDS-associated OIs has come from the improved use of drugs that had been developed previously to treat other infections or to treat OIs in individuals suffering from diseases other than AIDS. Few new drugs have been developed specifically to treat OIs in people with AIDS. For a number of OIs that are unique to or most commonly seen as complications of AIDS, there is little interest on the part of pharmaceutical companies to mount significant drug development efforts. The National Institutes of Health (NIH) funds researchers who generate much of the basic science information about the biology of the pathogens responsible for AIDS-associated OIs that provides a necessary foundation for successful drug development efforts. However, it is at present exceedingly difficult to advance basic laboratory findings to the stages of drug development, manufacture, and initial clinical evaluation. Should this situation continue, the Subpanel is concerned that the pace of development of new, more effective, and less toxic therapies to treat AIDS-associated OIs will be far too slow.

Future progress in preventing and treating AIDS-associated OIs will depend on progress in understanding the fundamental biology and pathogenesis of these diseases. To best accomplish this goal, investigators with expertise in microbiology, cell biology, genetics, and cancer biology should be encouraged to contribute to the study of AIDS-associated OIs. As with all other areas of AIDS research, there is a great need to attract and support young investigators in these areas.

Recommendations

To expedite progress in this area, the OI Subpanel offers the following recommendations:

1. Reinvigorate the basic science research effort on AIDS-associated OIs.

A. The highest priority of the NIH effort in OI research should be basic scientific studies of organism life cycles, metabolism, transmission, epidemiology, pathogenesis, and host response.

- The NIH should stimulate progress in these areas by support of investigator-initiated grants that encourage collaboration between basic research scientists and between basic research scientists and clinical investigators.

- The NIH should structure funding mechanisms in such a way to diminish the complexity of the grant application process (e.g., requirements that collaborators be at different institutions or that industrial collaborations be established) and maximize the process of scientific discovery.

- The NIH should encourage research into the pathogenesis of opportunistic diseases in their natural hosts and in relevant animal model systems.

- A program of small pilot grants should be implemented to support preliminary projects of high risk but significant potential promise. The intent of these grants (e.g., approximately $50,000 per year for 1 to 2 years) would be to test a principle or gather sufficient preliminary data for submission of an R01 grant application.

B. The NIH currently supports an active program of research in this area, and it is expected that the productivity of this effort will be enhanced significantly by the recommendations discussed elsewhere in this report, including those that call for the following:

- increased support for investigator-initiated research,

- expert peer review informed of the scientific priorities of the annual NIH Plan for HIV-Related Research,

- increased efforts to encourage young investigators to enter AIDS research, and

- increased efforts to attract established investigators with expertise in related areas to pursue AIDS-related research.

C. To further progress in this area, the Office of AIDS Research (OAR) and relevant Institutes, Centers, and Divisions (ICDs) should increase and better coordinate their efforts to foster research on AIDS-related OIs and continue to solicit the advice of non-Government scientists in identifying new research needs and opportunities in this area. As many of the AIDS-associated OIs also cause disease in individuals with other types of immunodeficiency and research on these pathogens is consequently supported with both AIDS and non-AIDS

funds, it will be important to view the NIH portfolio in this area as defined by scientific issue rather than funding mechanism.

- The OAR should establish an external expert advisory group to advise the OAR concerning evolving opportunities and needs in AIDS OI research. This group should review the NIH OI research portfolio on a yearly basis and recommend adjustments for programs that need to be updated or changed. This group should encourage improved reportability and accountability of programs sponsored by various ICDs. A consistent standard for high-quality science should be emphasized, and indicated resources should be redirected away from less productive programs. Many AIDS-associated OIs also cause disease in persons with other types of immunodeficiency, and research on these infections is supported with both AIDS-designated and non-AIDS funds; thus, the advisory group should monitor the NIH's OI research portfolio as a whole rather than only that portion supported by AIDS-designated resources. This approach will ensure better overall assessment and coordination of NIH's OI research activities.

- Existing NIH databases of funded research activities are inadequate for assessment of funds supporting OI research. The NIH should improve the data management and retrieval systems for ongoing research. The NIH data bases should be structured to permit greater ease of accessibility and be updated on a regular basis.

- The OAR, in collaboration with the relevant ICDs and industry representatives, should establish a regular review of OI research by sponsoring focus meetings on individual pathogens and on groups of related pathogens. This process should result in the development of goals that are jointly recognized by academic, Government, and industry scientists and will facilitate greater communication of relevant results and more rapid exploitation of research opportunities.

D. The NIH should take a more productive approach to training of scientists for the study of OIs. At present, the Subpanel believes the following:

- There is at present a crisis in the relative lack of incentives to enter the field of OI research and inadequate opportunities and support to train young investigators in OI research.

- The NIH should undertake to make it easier for young investigators to obtain initial NIH research support. Possible approaches might include a separate review process for R29 grants or more generous funding levels for these applications. Establishment of a program of short-term (1 to 2 years) grants to provide initial support to promising young scientists should also be considered.

- The NIH should continue to enhance training programs that encourage young scientists to pursue research on OIs. In addition, funding support should be considered for established investigators in one field to retrain in a laboratory studying opportunistic pathogens, and for young investigators within mentorship programs.

- The NIH should sponsor short courses (3 to 4 weeks) on opportunistic pathogens to attract young investigators as well as established investigators working in related fields to pursue research on AIDS-associated OIs.

2. The NIH should pursue innovative approaches to foster the transfer of new laboratory findings to early "proof of principle" clinical evaluation.

The OI Subpanel suggests the following strategies to accomplish this goal:

- The OAR should facilitate the translation of promising basic research findings to clinical applications. A translational research coordinator within the OAR, and informed by the external advisory panel on OIs described above, should work with basic scientists and industry to identify promising opportunities in their areas and help establish productive collaborative efforts.

- The OAR should work with the ICDs and the Small Business Administration to improve the quality and impact of AIDS-related research funded by the NIH through the Small Business Innovative Research (SBIR) grant program. These funds should be focused on important unmet needs in AIDS research through a new program of Request for Applications (RFAs) issued to small biotech or pharmaceutical firms. The topics of these RFAs should be defined by OAR and ICD staff members, in consultation with Government and non-Government scientists, to target emerging opportunities for the development of diagnostics and therapeutics for HIV infection and its associated complications.

- The NIH should strengthen the study sections that review SBIR grants related to AIDS with respect to scientific expertise and familiarity with contemporary scientific needs and opportunities, as articulated in the annual NIH Plan for HIV-Related Research.

- The Area Review Panel evaluating the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI) should consider the potential contribution of the program to identify and produce candidate agents for the treatment of OIs for which there is little or no interest within the pharmaceutical industry. Should the AIDS-related activities of the DTP program be continued in the future, they may provide a vehicle to advance candidate drugs from the research laboratory to a stage where an Investigational New Drug (IND) application can be filed with the Food and Drug Administration (FDA), permitting initial clinical evaluation. Should promising agents be identified in "proof of concept" clinical trials, it is likely that the interest of the pharmaceutical companies in further developing such agents may be increased substantially.

I. *Pneumocystis carinii*

A. Introduction

Pneumocystis carinii (PC) organisms are eukaryotic opportunistic pathogens that cause an often fatal pneumonia in immunocompromised hosts, especially persons with AIDS. Sequence comparisons of small subunit ribosomal RNA (srRNA), mitochondrial large subunit RNA (mtRNA), and other genes show PC to be more closely related to fungi than other protists. Its exact taxonomic position in the fungal kingdom remains controversial, and PC does not respond to common antifungal therapy. Although advances have been made in the understanding of the genetic organization of PC, knowledge of its basic biology remains rudimentary. The life cycle of PC has not been described. Likewise, the events leading up to infection in the mammalian lung have not been defined. The means of transmission has not been identified, although an airborne mode of infection has been supported by several animal experiments. An external amplification cycle remains a possibility. Despite advances in the prevention and treatment of *Pneumocystis carinii* pneumonia (PCP), the biology of PC and the pathogenesis and treatment of PCP continue to be essential research priorities.

- In the United States, PCP remains the most common opportunistic infection in adult and pediatric HIV-infected patients. Approximately 50 percent of adult patients and children aged less than 6 months had PCP as their initial AIDS diagnosis.

- Improved clinical management of PCP has resulted in decreased mortality (ranging between 15 to 40 percent), but there is still considerable morbidity associated with the infection as well as reduced quality of life due to accompanying symptoms (e.g., fever, dyspnea, loss of appetite, cough).

- Recurrent PC infections in AIDS patients are frequent, debilitating, and often the final cause of death. The management of the disease, requiring periodic visits to monitor drug toxicity and other complications, exacts its toll both financially as well as psychologically.

- When PCP occurs in HIV-uninfected persons, in whom the underlying immunosuppressive states are due to administration of corticosteroid or other agents to reduce transplant rejection or to treat underlying disease (e.g., Wegener's granulomatosis), a mortality rate higher than that in HIV-infected patients results.

- The primary risk factor predisposing to PCP in adults is a decrease in cell-mediated immunity, although there is some evidence that humoral immunity plays a role. The level of immunosuppression in HIV-infected individuals has been quantified by CD4 lymphocyte counts, and several clinical studies support the association of PCP diagnosis with CD4 counts below 200/μl in patients not receiving PCP prophylaxis. In contrast, PCP may develop in children with CD4 counts well above 200/μl, suggesting that other immune defects may also influence the course of this infection.

- No PCP therapy has been found to be completely curative. The most efficacious therapy for PCP is treatment with trimethoprim-sulfamethoxazole (TMP-SMX), although adverse side

effects associated with its administration (toxicity, rash, nausea, and vomiting) resulted in discontinuation more frequently than other standard treatments with pentamidine or dapsone.

- The risk of developing PCP in persons receiving preventative therapy is markedly reduced, but it is not eliminated. CD4 lymphocytopenia is the main predictor for failure of PCP primary or secondary prophylaxis, regardless of treatment regimen. The prophylactic failure rate associated with the most common anti-PC therapies (TMP-SMX, pentamidine, and dapsone) is approximately 20 percent. While no difference was observed in failure rate among primary or secondary prophylaxis, the median time to death after start of prophylaxis was 2.0 years for primary prophylaxis and 1.2 years for secondary.

B. Transmission and Epidemiology

Today, PC is the most frequent OI associated with HIV-infected individuals in developed countries. Many questions remain about the basic biology and natural history of PC infection, due largely to the lack of a continuous *in vitro* culture system. The life cycle has not been defined, and the source of infection is not known. In AIDS patients, a primary PCP infection is often followed by a series of recurrent PCP infections. The source of primary versus recurrent PCP may be distinct. Possible sources of infection include a direct transmission from host to host, infection via an external cycle that produces the infective particle, or a combination of both. Host-to-host transmission could involve subclinically infected carriers or persons with fulminant PCP as the transmitting populations, and nonimmunocompromised populations as the recipients. Drug-resistant organisms remaining after therapeutic or prophylactic treatments may possibly contribute to recurrent infections.

Improved epidemiologic tools are needed. Serologic markers would be useful for discrimination between acute-phase infection or a remote exposure.

Genetic typing of PC isolates would provide the necessary tools to assess these major epidemiologic questions. Animal models of PCP have provided much of what is known about the airborne mode of transmission, the genetic structure of PC, and other aspects of its biology. Molecular typing of human PC isolates has begun, but the field is limited by the number of sequenced genes available that provide sufficient discriminating power.

Lack of knowledge concerning the origin of PCP in AIDS and other immunocompromised populations impedes the clinical management of the infection. The inability to assess resistance in PC populations further exacerbates the problem.

Outstanding Research Needs

The Subpanel recommends support for basic molecular biology, transmission, and epidemiology studies. Basic tools of molecular biology must be developed to aid in transmission and epidemiologic studies. These tools include, but are not limited to, targeted gene sequence determinations, transformation systems, and expression systems in heterologous organisms. Animal models can be used for controlled transmission and epidemiologic studies, but studies on human PC are encouraged. The NIH should coordinate planning of epidemiologic studies of

human PC with other Government agencies, such as the Centers for Disease Control and Prevention (CDC).

C. Pathogenesis

Immunopathogenesis

The roles of the host immune responses that predispose to infection and operate during the course of infection are not well understood and may, in some cases, be problematic in the resolution of infection. The specific host immune defects that predispose individuals to PCP have not been definitively elucidated. Impaired cellular immunity has been considered to be the major factor, due to the frequency of PCP in AIDS patients, most of whom have impaired cellular immunity. A role for humoral immunity has been suggested by observation of PCP in patients and experimental animals with B-cell defects.

Until recently, it was thought that the host immune response was not involved in the pathogenesis of PCP; however, inflammatory response in AIDS patients with PCP shortly after administration of anti-PC therapies leads to decreased blood oxygenation. Subsequent amelioration of this response with corticosteroid therapy suggests a deleterious effect, at least in some situations.

Anatomic and Physiological/Biochemical

It is presumed the infection is established by inhalation of infectious airborne particles. The dose of PC necessary to induce infection is not known, nor is the time period in which the infectious particles can remain in the immune-competent or -incompetent host lung. Evidence of colonization in immune-competent individuals would suggest mechanisms for circumvention of the immune system and would imply a more virulent nature than what is currently recognized for this organism. Early in the infection, attachment of the trophic form of the organism to the Type I pneumocytes is observed. The nature of this interdigitation and its role in initiation of infection are poorly understood but, if necessary, could provide a target with which to prevent infection. Host requirements for the establishment of infection or nutrients needed to sustain PC in the lung milieu have not been defined.

Changes in the alveolar environment have been shown to include anatomic and biochemical alterations. Alveolar capillary membranes have increased permeability, degenerative changes in lung cells are apparent, and other changes indicative of diffuse alveolar injury slowly evolve during the course of the infection. The major physiologic/biochemical manifestations include impaired gas exchange, altered lung compliance and mechanics, and a decline in surfactant phospholipids. Without treatment, these factors lead to respiratory insufficiency and death, due to severe lung injury.

Outstanding Research Needs

- Basic studies elucidating the beneficial or deleterious roles of the host immune responses directed against PCP, the effects of the organism on lung physiology, and basic research for antigenic and serologic reagents are recommended. The development of immunotherapies and vaccines should proceed more rapidly based on the results of these studies. Research

leading to a clearer understanding of the establishment of infection (e.g., the role of organism attachment to host cells) is recommended.

- The pathology that PC exerts on the host remains poorly understood, yet the development of effective prophylaxis or therapeutic measures will likely be greatly expedited by understanding better the response of both the immune-competent and immunosuppressed hosts to the organism.

- The Subpanel recommends that research leading to a clearer understanding of the establishment of infection (e.g., the role of organism attachment to host cells) be emphasized.

- The Subpanel recommends supporting research to develop a noninvasive diagnostic test that would discriminate between acute-phase infection, remote infection, or exposure.

Rationale

It is not yet clear what roles the host immune system plays in the prevention and exacerbation of the disease state of PCP. Before immunomodulatory prophylaxis or therapeutic measures are proposed, it will be necessary to understand better the response of the immune-competent host and immunosuppressed host to the organism. Likewise, the pathology PC exerts on the host remains poorly understood.

D. Diagnosis

Patients with PCP confirmed by cytological techniques have been shown to reach better clinical outcomes than those empirically treated. PC cannot be continuously cultured, and the diagnosis of PCP relies on microscopic detection of the organisms in biological fluids. Sputum induction is a rapid and cost-effective means to sample patient populations, provided that appropriately trained technicians are available and patients have sufficient organism burden for detection. Bronchoalveolar lavage (BAL) is a safe and effective approach for obtaining samples from AIDS patients and other immunocompromised patients. A number of staining techniques are useful for organism identification:

1. Papanicolaou

2. giemsa or a rapid variant

3. methenamine silver, and

4. immunofluorescent kits based on monoclonal antibody detection or use of certain polyclonal antisera.

Polymerase chain reaction (PCR)-based methods using targeted amplification to single gene sequences or nested applications have been reported. There are concerns, however, about false-positive results and the importance of detecting PC DNA rather than organisms in interpretation of disease or infection. The development of quantitative PCR methods, especially those

employing RT-PCR to determine viability, may be useful for assessment of efficacy throughout the course of therapy or for detection of latent or drug-resistant organisms.

Outstanding Research Needs

- Development is needed of a rapid noninvasive diagnostic test that would discriminate between acute-phase infection and remote infection or exposure.

Rationale

A serologic test antigen-detection method or nucleic acid-based assay that would discriminate between active infection versus previous exposure would be less invasive than currently available diagnostic methods and would permit an assessment of the patient's phase of infection.

E. Therapy and Prophylaxis

The most efficacious therapies for the treatment of PCP are TMP-SMX and pentamidine. Neither appears to be cidal to PC, and, once administration is stopped, the infection may recur. Apparent in the treatment of PCP in AIDS patients is the frequency of recurrent episodes. These recurrent infections may arise from lack of pneumocysticidal activity by the compounds, drug-resistant organisms, reinfection through exogenous sources, poor compliance with treatment, poor absorption of the compound, or the inability of the host to clear the resolving infection. The adverse reactions associated with TMP-SMX reduce its utility for some AIDS patients, and treatment failures, while less commonly seen than with pentamidine, nonetheless occur. Experimental drugs, some of which have reduced adverse reactions, have not been found to be more efficacious. Consideration of these factors suggests a multifactorial treatment of PCP. There is a need to identify effective, well-tolerated anti-PC compounds and to consider augmentation of the host immune system as a factor for organism clearance and resolution of infection.

Outstanding Research Needs

- The Subpanel recommends support for the development of therapeutic and prophylactic modalities. A goal should include an identification of a battery of agents, including combinations of chemotherapeutic and immunotherapeutic agents, to prevent the emergence of drug-resistant organisms.

- Research is needed to develop strategies to detect drug resistance and identify mechanisms of resistance used by the organism.

- It is critical to revise current mechanisms for introducing candidate compounds identified through drug-screening programs into safety and tolerance trials followed by efficacy evaluation. Suggestions include an OAR-affiliated translational research coordinator working with expert external advisors to select promising drugs from basic OI research for clinical development and to oversee the early transition of those candidate agents into efficacy evaluations.

Rationale

Definition of the life cycle, transmission, and epidemiology of PCP is essential for therapeutic strategies as well as prophylaxis. Basic biological studies of PC metabolism should provide potential drug targets.

II. Fungal Infections

A. Introduction

Fungal infections can occur throughout the clinical course of HIV disease and are responsible for significant morbidity and mortality in HIV-infected persons. Fungal infections also are a significant threat to individuals with other types of immunodeficiency, and this problem is further increasing in importance as aggressive cancer chemotherapy regimens and both bone marrow and solid organ transplantation become more widely used. Although a number of drugs are available to treat specific fungal infections, none is completely satisfactory or optimally effective in the setting of severe immune system compromise. Some standard antifungal therapies are limited by increasing levels of drug resistance. Development of more effective approaches to prevent or treat serious fungal infections will require a more complete understanding of the biology and pathogenesis of these organisms.

A focus on fungal infections should be encouraged as an essential basic science agenda for the NIH:

- Data on incidence and prevalence of fungal infections are incomplete and frustrate accurate delineation of the magnitude and changing character of risk populations and their infections.

- Rapidly fatal fungal infections require that rapid and accurate diagnoses be made. However, the current technologies used to diagnose fungal infections rapidly and optimally guide treatment suffer from significant limitations and, as a result, the need for empiric antifungal agents is commonplace.

- Success in curing and/or preventing deep mycoses in patients with AIDS and other immunocompromising conditions remains severely limited with present therapies.

- Studies of the molecular pathogenesis of fungal infection could lead to translational application, e.g., development of more effective drugs or vaccines.

B. Epidemiology

Since fungal infections are not reportable diseases, data on incidence and prevalence of these infections are not readily available to identify the magnitude and changing character of risk populations.

Outstanding Research Need

- Serial studies of the incidence, prevalence, and clinical course of at-risk patients of different age groups for a variety of fungi such as *Candida, Pneumocystis, Cryptococcus, Aspergillus, Histoplasma, Coccidioides,* etc., are necessary.

Rationale

This information is important to allocate resources properly for prevention, diagnosis, and treatment strategies.

C. Pathogenesis

Studies of molecular pathogenesis of fungal infection should be encouraged as an essential basic science agenda for the NIH. Research progress in this area has great potential for advancing our understanding of the pathobiology of fungal infections and could lead to important translational applications such as the development of drugs or vaccines. Although there are many avenues for research in this area, the following topics may be particularly rewarding:

a. Identification of virulence determinants

b. Delineation of immunogens/superantigens

c. Study of mechanisms of morphologic switching

d. Adhesions

e. Incorporation of genetic systems with related organisms for study of virulence and/or morphogenesis: e.g., *Saccharomyces* genetics for studies of *Candida* and possibly other yeast pathogens; direct studies of the fungal pathogen, rather than extrapolation from model systems with nonpathogenic fungi, should be encouraged

f. Genome sequencing of *Candida/Cryptococcus/Aspergillus* insofar as federally funded efforts do not duplicate those undertaken by industry.

Outstanding Research Needs

- Investigations of the basic molecular pathobiology of fungi should be encouraged and supported. It will be important to attract molecular biologists currently conducting research in other eukaryotic systems to interact with mycologists who understand clinical disease.

Rationale

Through molecular studies of pathogenesis, directed drug and vaccine development can be realized.

D. Diagnosis

Routine culture techniques are not sufficient either for the diagnosis of rapidly fatal fungal infections, including *Candida* and *Aspergillus*, or for the ability to determine colonization from infection in immunocompromised hosts. This lack of sensitivity and specificity requires empiric antifungal use in certain patient populations. Although this problem of rapid and accurate diagnosis may be of less concern in AIDS, it has significant implications in many immunocompromised hosts.

Outstanding Research Needs

- The NIH should encourage development of reliable methods for the rapid diagnosis of infection using nucleic acid or protein-based detection strategies and should coordinate this work with industry.

Rationale

There is a need for the institution of rapid, pathogen-specific therapy once fungal infections take hold in immunocompromised hosts.

E. Treatment and Prophylaxis

Success in curing and/or preventing deep mycoses in patients with AIDS and other immunocompromising conditions remains severely limited with present therapies. The following three general categories list specific areas of important research focus:

Drug development

a. Molecular mechanisms of resistance to azole antimicrobials

b. Epidemiology of drug resistance

c. Use of structural biology to identify drug targets

d. Treatment of refractory thrush and vaginitis in AIDS patients

e. Improved methods for the treatment of cryptococcal meningitis in AIDS patients as a model for eradication of infection in deep mycoses

Vaccines for the prevention of fungal infections: histoplasmosis, cryptococcosis, candidiasis, coccidioides

a. Identification of potential immunogens in relevant fungi

b. Characterization of mechanisms of immunity

c. Participation of relevant cells (e.g., Th1 or Th2 lymphocytes) and cytokines

Immunotherapy

a. Use of relevant animal models to identify fungicidal cells and cytokines applicable to human infection.

b. Serial studies of the evolution of immunity in patients of different age groups (e.g., to *Candida, Pneumocystis,* etc.)

c. Utility of monoclonal and polyclonal antibodies or peptides in inhibiting steps in pathogenesis as an alternative to active immunization

d. Strategies for the enhancement of the immune response in AIDS patients infected with fungal pathogens

Outstanding Research Needs

- The NIH should support basic science initiatives to identify drug targets, drug-resistance mechanisms, animal models, clinical strategies of treatment and prophylaxis, and immune modulation.

Rationale

Better treatments are needed to achieve a high percentage of cure in AIDS patients and also to circumvent drug-resistance problems. Prevention of mycoses in an identified high-risk population is an important goal.

III. Viral Infections

A. Introduction

A number of viral infections complete the clinical course of HIV disease. Although appreciation of the nature and importance of these infections has increased recently, current methods to treat or prevent important viral diseases are inadequate.

Viral OIs include diseases due to productive viral replication as well as virally induced cell proliferation. These OIs typically affect human beings as their sole host throughout the course of an HIV infection as well as during certain courses of immunodeficiencies (e.g., in bone marrow transplant recipients). Viral OIs can be categorized by their impact upon the AIDS-affected population and, thus, their priority for NIH AIDS-related investigation:

The investigation of the pathogenesis of these diseases is an essential priority for the NIH:

- Viral OIs can have significantly different clinical presentation in persons immunosuppressed due to HIV infection as compared with persons immunocompromised due to other courses of disease or therapies.

- Diagnostic tests to detect viral OIs are not predictive of disease.

- Vaccine strategies for viral OIs currently at various stages of development need to be assessed for their efficacy and safety in treating HIV-infected individuals; studies of the safety, immunogenicity, and efficacy of these vaccines can, in turn, provide data on how the progression of immunodeficiency modifies the effectiveness of vaccination.

- The epidemiology of viral OIs in different AIDS-affected populations remains incompletely defined.

  High Priority: Disease arises in a significant or growing proportion (>10 percent) of AIDS patients, with significant morbidity and mortality: cytomegalovirus (CMV), Kaposi's sarcoma (KS) herpesvirus (KSHV, also known as HHV-8), Epstein-Barr virus (EBV).

  Intermediate Priority: Disease arises in a lower proportion (<5 percent) of AIDS patients and/or is controllable: herpes simplex virus (HSV), varicella zoster virus (VZV).

  Low or Unclear Impact: Low incidence of disease or unclear epidemiology in AIDS patients: HHV-6, HHV-7, JC virus (JVC), papilloma virus (HPV), hepatitis B, C, or G viruses (HBV, HCV, HGV).

B. Epidemiology/Relevance to AIDS and non-AIDS Immunocompromised Individuals

It is striking that the epidemiology of viral OIs in different AIDS-affected populations (adult heterosexual, adult homosexual, and pediatric) remains incompletely defined. For high- priority viruses, disease incidence in pediatric populations needs to be studied. For intermediate- or low-impact viruses, particularly HHV-6, HHV-7, JCV, and the hepatitis viruses, greater attention to identifying the contribution of these viruses to disease is needed. For all groups of viruses, the impact within the AIDS-affected population needs to be compared with the impact in immunocompromised individuals exclusive of AIDS (all viral OIs) or in cancer patients (EBV, KSHV). Any balance in research support for a particular viral pathogen must take into account the total funding received as a result of the overall (AIDS and non-AIDS) medical impact of a particular pathogen.

Related to epidemiology (molecular epidemiology), the role of KSHV in the etiology of KS remains to be firmly established, and the role of EBV in certain lymphoproliferative disease requires further investigation. The association and general distribution of KSHV among the general population require additional study.

C. Pathogenesis of Viral Disease

Viral OIs typically affect humans as their sole host and often the relevance of animal models of disease is unclear. Investigation of pathogenesis of these diseases must include comparative work in the patient as well as work with available animal and cell culture models of latency, reactivation, persistence, and acute replication and must include studies aimed at understanding diseases that result from direct viral damage as well as immunopathological processes. AIDS-associated viral diseases result directly from reactivation of latent virus (CMV, HSV, VZV, JCV, HBV/HCV) or the outgrowth of proliferating latently infected cells (KSHV, EBV, HPV). The viral and host (immune) determinants of persistent or latent infection in the immunocompetent host need to be understood, and the viral and host determinants allowing reactivation or outgrowth during progressive immunosuppression need to be determined. It is striking that viral OIs, such as CMV, can have significantly different clinical presentation in persons immunosuppressed due to HIV infection as compared with other causes of immunodeficiencies (such as in bone marrow transplant recipients). Natural cellular and cytokine as well as adaptive humoral, cellular, and cytokine mechanisms need to be understood in the context of individual viral infections. Peculiarities in the immune deficit occurring in AIDS need to be investigated in the context of viral immune control mechanisms.

Virulence determinants

Better understanding is needed concerning which viral functions permit establishment of infection and lead to disease. Funding of these investigations should follow NIH Institute focus (NIAID, general pathogenesis; NCI, cell proliferation; other Institutes, appropriate organ-specific diseases)

1. CMV, HSV, VZV: Identify and understand mechanisms of viral latency, including the viral and host functions that restrict viral productive replication. Investigate viral genes controlling dissemination, tissue invasion and disease following reactivation, and the relationship of disease to immunodeficiency. Investigate host cell functions that restrict intracellular viral replication. Understanding these functions will provide additional targets for antiviral therapy and vaccine development. Investigate mechanisms of drug resistance and the impact of drug resistance mutations on pathogenesis in animal models and in patients.

2. KSHV: Identify viral genetic determinants causing cell proliferation and their effect on the host cell. Determine the cellular and molecular events that lead to proliferation and the cell types involved.

3. EBV: Understand the mechanisms of EBV-induced lymphocyte proliferation. Studies should be aimed at the function of latent gene products and their interaction with cellular signaling pathways as well as on ways to use this information to control proliferation and understand the particular determinants associated with lymphoproliferative disease.

4. Other viruses: Investigation of the viral and host genetic basis of how viral persistence, immune response, or the damage to specific tissues leads to disease.

Immune control

1. Understanding mechanisms of immune control

   a. productively infected cells: CMV, HSV, VZV, HHV-6, HHV-7, JCV, hepatitis viruses

   b. latently infected cells: CMV, KSHV, EBV, HPV, HSV, VZV, HHV-6, HHV-7, JCV, hepatitis viruses

   c. proliferating cells: KSHV, EBV, HPV

2. Identification of viral antigen targets of immune control. Determine the spectrum of antigens that are required for the induction of protective viral immunity

3. Understanding the contribution of the host immune response to disease (immunopathology)

D. Diagnosis

Current diagnostic tests that detect viral OIs are not predictive of the presence of disease.

Outstanding Research Needs

- More work on the patterns of viral gene expression or antibodies needs to be completed in order to have diagnostic tests that are predictive of disease and that can be used to make informed choices in therapy. Diagnostic tests need to be developed that can be applied to readily accessible cells or fluids. Development of rapid standardized methods to identify infections with drug-resistant viruses is needed.

E. Prophylaxis and Treatment

Vaccine strategies for CMV, HSV, EBV, and HCV are in various stages of development. Vaccines are currently in universal use for VZV and HBV, but how effective they will be in protecting HIV-infected individuals from these viruses is unknown. Most vaccines in development could be investigated for ability to protect HIV-positive individuals but the potential efficacy of vaccines to induce protective immune responses in the setting of HIV infection is uncertain. Further understanding of immune targets responsible for maintaining latency and preventing cell proliferation could be adopted by vaccinologists interested in providing protection during immunodeficiency. Further support is needed to ensure that effective vaccines proceed into general use in the population. The major difficulty with all vaccines is the expectation that progressive immunodeficiency will complicate vaccination. Thus, reactivation or reinfection of viral OIs may remain a long-term problem in AIDS patients. (An understanding of the impact of vaccination on OIs will emerge first from studies of HBV and VZV vaccines.) Excellent therapeutics for HSV are available; however, long-term use in AIDS patients leads to drug resistance. There are few alternative drugs available to combat drug-resistant virus. Therapeutics for CMV and VZV exist but are not as effective as for HSV. Major pharmaceutical

focus on CMV may bring additional therapeutics to bear on diseases caused by this virus. For the proliferative disease caused by KSHV and EBV or chronic viral diseases caused by JCV, HBV, HCV, either poor or no active therapeutic agents are available. More emphasis by NCI on therapeutics directed at virus-induced proliferative diseases is warranted.

Vaccines

1. CMV (live attenuated versus subunit): This virus has over 200 potentially immunogenic proteins, and the subunit vaccines farthest along in development are derived from the Gb protein. Live attenuated virus has been tested (Towne), but it may be over-attenuated. Towne vaccine showed a marked benefit to solid organ transplant recipients, who are immunocompromised. More evaluation of virulence determinants may enable thoughtful construction of live attenuated viruses. There is necessarily a greater emphasis placed on testing candidate vaccines in humans because appropriate animal models for infection or disease protection do not exist.

2. KSHV: Currently no vaccines are available. However, from the impending release of the viral genome sequence and growth of the virus in tissue culture, subunit strategies may emerge.

3. HSV (live attenuated versus subunit): Subunit vaccines with one (Gd) or two (Gd, Gb) viral proteins have reached Phase III study in humans. Strategies for attenuating live virus also exist but are less well developed. Animal models for HSV infection exist, but the predictive value of immune mechanisms in animals has been questioned. Thus, a greater emphasis on testing candidate vaccines in humans seems appropriate.

4. EBV (subunit): The major viral glycoprotein (gp220/350) is immunogenic and forms the basis for development of a vaccine for this difficult-to-propagate virus. There is no biomedical precedent to predict whether this antigen will be sufficient to prevent infection and proliferative diseases in HIV-infected persons.

Therapeutics

1. CMV: Additional options for therapy are needed. Ganciclovir and foscarnet are both too toxic, and drug-resistant virus arises during maintenance therapy. Cidofavir, a nucleotide analogue targeted at the viral DNA polymerase, and DRB-related molecules targeted at UL89 are promising drugs at different stages of development. The maturational protease (UL80) has been chosen as a target by many groups in industry. Many pharmaceutical companies have taken an interest in CMV. There is a need to better understand viral functions that might be targets of rational drug design.

2. KSHV and EBV: It is unclear how antivirals that target replication functions can be activated against the malignancy associated with these viruses once key cellular transformation events have taken place. The NIH should support research on the development of antivirals targeted at the latency or proliferative functions encoded by these viruses.

3. HSV/VZV: Acyclovir is a successful antiviral, and many AIDS patients use it prophylactically because of preliminary indications of a benefit. Penciclovir is similar to acyclovir. A backup for acyclovir is needed.

4. Other OIs: There are no therapeutics available for HPV, JCV, or the hepatitis viruses.

IV. Mycobacterial OIs

Mycobacterium tuberculosis (MTB) and the nontuberculous mycobacteria, particularly *Mycobacterium avium* complex (MAC), are frequent infectious complications of AIDS. Although there are some areas of overlap in the microbiology and basic understanding of the manifestations of the diseases mycobacteria cause, they differ in important ways in their public health importance, their basic pathogenic mechanisms and properties, and their susceptibility to antimycobacterial drugs used to prevent or treat disease. Separate consideration, therefore, seems warranted.

Mycobacterium tuberculosis

A. Introduction

MTB differs in two substantive ways from the other major infectious complications of AIDS. First, it is not strictly an OI but is rather a virulent pathogen that causes morbidity and mortality in HIV-uninfected as well as HIV-infected individuals. Second, MTB is a particular public health concern as it is the only infectious complication of HIV that can be transmitted by the respiratory route to others. This contributes to the rising incidence of tuberculosis (TB) worldwide and nosocomial outbreaks of disease.

B. Epidemiological Considerations

TB is the leading cause of death worldwide due to an identifiable infectious agent. As it affects individuals in the most productive years of life, it also has tremendous social, economic, and political importance. The decades-long decline in incidence of TB in the United States and many other developed countries was reversed in 1985. HIV is one factor in this disturbing trend, but homelessness, poverty, and immigration from geographic areas of high prevalence are equally relevant. In fact, TB in immigrants to the United States and the potential for infection of U.S. citizens abroad clearly means that TB cannot be controlled in the United States without attending to its control in other countries. Overall, 90 percent of TB cases and 95 percent of TB deaths currently occur in developing countries.

HIV attacks the CD4 lymphocytes that are critical to the host immune response against MTB. As a consequence, HIV is the greatest risk factor known for reactivation of a latent MTB infection and progression of recent infection. In 1994, an estimated 5.6 million individuals were coinfected with TB and HIV. The highest prevalence of coinfection occurs in sub-Saharan Africa and Asia. The impact of HIV on TB in a country is determined by the coprevalence of these pathogens. For example, in countries endemic for MTB infection and an HIV prevalence of 20 percent, TB can be expected to increase 7.5-fold.

Worldwide, in developing as well as developed countries, the incidence of TB is increasing. It is estimated that there will be 10.2 million new cases in the year 2000 and that 14.8 percent of these will be among HIV-infected individuals. Besides affecting the incidence of TB, HIV has a dramatic effect on its natural history. After exposure to a case of infectious TB, 40 percent of HIV-infected persons will develop active TB within 4 months. HIV, therefore, markedly increases the attack rate and compresses the period between infection and disease. As a consequence, prevalent strains including multidrug-resistant organisms may be spread rapidly within populations and institutions. Nosocomial outbreaks characterized by a high attack rate in HIV-infected individuals, high mortality, and rapid progression to death have occurred in the United States.

The increasing incidence of TB in the United States and spread of multidrug-resistant TB (MDR-TB) led the U.S. Public Health Service (PHS) to develop an Interagency Task Force and a National Action Plan to combat MDR-TB. Funding in the United States for basic research on TB increased by a factor of 10, and unprecedented resources were committed to TB control in New York City. The result has been a decline in incidence of TB in New York City and throughout the United States in 1994, although at a cost not affordable elsewhere ($40,000 per case averted).

In March 1993, the World Health Organization (WHO) declared TB a global public health emergency. TB is the only disease ever so designated and represents a threat to both HIV-infected and uninfected people worldwide. The impact of TB on HIV morbidity and mortality is huge. The WHO estimates that 44 percent of all HIV deaths are due to TB. The number of MTB/HIV coinfected individuals in a country and in at-risk groups will determine the local impact. In the United States, TB was the AIDS indicator condition in 5.0 percent of 79,674 adults and adolescents (1994 CDC data). A study of prevalent infections prior to death in 1,883 HIV-infected persons followed by the NIH Community Program for Clinical Research on AIDS (CPCRA) during the period 1990-1994 showed that 7.7 percent males and 11.5 percent females developed TB. The proportion of TB was significantly higher in Latinos (11.4 percent) and African-Americans (13 percent) than whites (4.3 percent); injection drug users (IDUs) (15.2 percent) than non-IDUs (5 percent); the Northeast (20.4 percent) compared with other regions (3.3 to 6.8 percent). In the Northeast, TB was more prevalent that MAC (12.3 percent), and its frequency ranked only behind PCP, wasting syndrome, and CMV.

C. Clinical Needs

The available means of diagnosis, prevention, and treatment of TB are outmoded and inadequate in the best of circumstances. In fact, it is the lengthy duration of treatment (at least 6 months) that drives up the cost of TB control and the associated noncompliance that fosters the development and spread of drug-resistant strains.

MDR-TB is exorbitantly expensive to treat ($250,000/case), and available drugs produce cure in only about 50 percent of MDR-TB-infected patients.

TB in HIV-infected persons presents particular problems in diagnosis, prevention, and treatment. The manifestations of TB in HIV-infected persons may be atypical either in terms of the nature of the pulmonary process (lower lobe, noncavitary infiltrate; pleural disease; hilar or mediastinal adenopathy) or sole involvement of extrapulmonary sites. The insensitivity of available

diagnostic modalities and the danger of nosocomial transmission have led to a policy of isolation of suspect cases until TB can be reasonably excluded. This expensive treatment further encumbers care of the HIV-infected patient. As regards prevention, live attenuated vaccines are not ideal for persons that are infected with HIV. Preventive chemotherapy is a logical modality, given the great risk of progression from MTB infection to TB; but it is not known whether lifelong treatment is necessary, and preventive therapy may be difficult to impossible to provide in developing countries. It also is unclear whether treatment of drug-sensitive TB needs to be maintained for life. Lethality of TB in the HIV-infected individuals is high in part because TB accelerates HIV replication and the course of immunodeficiency. It is not known whether this process can be ameliorated by tumor necrosis factor (TNF) inhibitors or antivirals. Treatment of MDR-TB in the setting of coexisting HIV infection is made more difficult because HIV-infected persons also infected with MDR-TB often progress rapidly to death.

D. Scientific Opportunities

Basic fundamental advances in molecular biology, immunology, microbiology, and biochemistry promise to yield insights for improved diagnosis, prevention, and therapy of TB. There has been a rapid expansion of funding of basic research on TB, most of it supported by NIAID and more recently by the National Heart, Lung, and Blood Institute (NHLBI). Through June 1995, NIAID's Division of Microbiology and Infectious Diseases (DMID) lists 109 projects. These include a contract to Colorado State University for production of MTB antigens, a contract to Colorado State University and the University of Texas for testing vaccines in animal models, and a contract to Case Western Reserve University to translate the basic advances to clinical application. NIAID's Division of AIDS (DAIDS) has supported a drug discovery program. Phase III trials of chemotherapy and preventive therapy have not been conducted successfully through domestic HIV trials networks, such as AIDS Clinical Trials Group (ACTG) and CPCRA, as compared with international sites.

The venue of basic and translational research has been expanded through RFAs, Request for Proposals (RFPs), and unsolicited R01s, mostly through NIAID and more recently through NHLBI. Plans have not been developed for continuation of these projects and programs, however, and the rate of success of competitive renewals evaluated by regular study sections has been dismal.

Outstanding Research Needs

- TB research must be accorded the highest priority by the NIH. TB is not simply a U.S. problem or an HIV-related problem. TB cannot be controlled in this country until it is controlled worldwide. The current level of NIH funding of TB research is appropriate given the public health importance, clinical needs, and the scientific opportunities.

- To optimize research efforts on TB, increased coordination is required at multiple levels: within the PHS (NIH and CDC), among the NIH Institutes, and within NIAID (DMID, DAIDS) as well as with other funding agencies (WHO). This coordination must be sustained and enhanced, independent of the current high level of public and political interest.

- The NIH should give special consideration to ensuring that successful research projects and programs are sustained. The flurry of RFAs and RFPs has galvanized the scientific community. This impressive effort will, however, stall unless special consideration commensurate with the importance of this topic is accorded to the review process for competitive renewals.

- Scientific areas of particular importance include studies of fundamental mechanisms of drug resistance and drug action, new drug targets, latency, and protective immunity. The NIH should give a high priority to basic and applied research on TB vaccine development.

- To increase our understanding of TB and develop new drugs, a comprehensive effort is essential to address and resolve the disincentives to pharmaceutical companies, given the low incidence of TB in the United States.

- The NIH should redirect major clinical trials efforts to international sites in areas of high incidence of TB (and HIV).

Rationale

The current NIH research priorities are appropriately centered on basic research rather than clinical trials. A steady effort and commitment must be made, however, to catalyze and ensure translation of this research into improved means to prevent, diagnose, and treat TB. This ultimately should include support of international sites capable of conducting Phase III clinical efficacy and vaccine trials.

Mycobacterium avium

A. Introduction

Mycobacterium avium complex (MAC) is the most common disseminated bacterial infection in patients infected with HIV and also is increasing in importance as a cause of pulmonary disease in HIV-uninfected populations.

B. Epidemiological Considerations

Disseminated MAC is a late complication of HIV, usually occurring when the CD4 count is less than 75/ml. It has been increasing in incidence among AIDS patients for a variety of reasons. With the recognition that MAC is pathogenic and with the availability of effective chemotherapeutic agents, the diagnosis is more likely to be established antemortem. A large factor is the increased use of prophylaxis against *Pneumocystis carinii*. A subcohort analysis of 844 HIV-infected individuals showed that MAC disease developed in 33.4 percent of those who received early PCP prophylaxis and 17.3 percent of those who did not. In a study of the occurrence of OIs in HIV-infected persons, MAC was a complication of HIV infection in 25 percent of persons and ranked second only to PCP. MAC occurred more commonly in the West and Southeast than the Northeast, and in non-IDUs compared with IDUs. For unknown reasons, disseminated MAC is not evenly distributed worldwide. It is, for example, infrequent in sub-Saharan Africa.

Both pulmonary MAC and disseminated MAC are now also occurring more commonly in HIV-uninfected persons without underlying diseases, although the prevalence is far less than in the presence of HIV infection.

C. Clinical Needs

The development of drugs of the azalides class provided a potent new modality for the treatment of disseminated MAC disease. Acquired resistance to these agents is, however, a problem. Optimal regimens for treatment are not known. Unfortunately, disseminated MAC occurs so late in the course of immunodeficiency that even effective therapy for MAC has only a meager effect on survival and quality of life. Both rifabutin and clarithromycin are effective in preventing MAC. Breakthrough infections seen in patients being treated preventively with clarithromycin often result from drug-resistant organisms. Prophylaxis has been recommended by the PHS but appears to be underapplied.

D. Scientific Opportunities

Basic research on MAC has lagged behind MTB in part because of greater difficulty in the genetic manipulation of this organism (e.g., transformation) and in part because of the uncertainty concerning its clinical role and, more recently, the availability of drugs with high activity. In general, less is known about the basic biochemistry, molecular biology, protective immunity, and pathogenesis of MAC as compared with MTB. As contrasted with TB, pharmaceutical interest in MAC has been higher and clinical trials have been conducted successfully within the ACTG and CPCRA.

Although program support has been possible through NIAID's National Cooperative Drug Discovery Group for the Treatment of OIs (NCDDG-OI), the number of R01s supporting basic fundamental research on MAC is inadequate.

Outstanding Research Needs

- The NIH should convene a consensus panel to review current research activities and scientific opportunities concerning MAC. The NIH should develop an expanded scientific agenda through RFAs and Program Announcements.

- The NIH should encourage research in the areas of protective immunity, pathogenesis, mechanisms of drug resistance, and identification of new drug targets.

- The NIH should address the issue of protective immunity in humans through epidemiologic studies in areas of high and low prevalence of disseminated MAC in AIDS patients.

V. *Toxoplasma gondii*

A. Introduction

Toxoplasmic encephalitis (TE) is the most common cause of focal central nervous system (CNS) infection in the HIV-infected patient. All HIV-infected individuals latently infected with *Toxoplasma gondii* are at risk for development of reactivated disease in the form of TE.

In reviewing the priority recommendations from the previous NIH-sponsored Workshop on Future Directions in Discovery and Development of Therapeutic Agents for Opportunistic Infections Associated With AIDS, two conclusions were readily apparent:

1. Fundamental progress has been made in many of the areas previously targeted for funding, and support from DAIDS has been instrumental in many, if not most, of the advances. Hence, the concept that targeting specific areas for research and directing funds to these areas will facilitate progress seems to be borne out in the area of *Toxoplasma* research.

2. However, many of the fundamental questions have not been solved. Key issues that either are being addressed currently or remain to be approached cannot and should not be downgraded when new or existing groups apply for funding or renewal in these areas.

B. Epidemiology/Relevance to AIDS

Currently, in 23 percent of those individuals who develop TE in the United States, this infection is the AIDS-defining diagnosis. In a recently reported study of U.S. and French patients not receiving primary prophylaxis, 33 percent with a CD4 count of <50 cells/μl developed TE within 12 months, and 45 percent developed TE within 18 months. Since it is estimated that up to 30 percent of the population in the United States is latently infected, the population at risk is substantial.

A large, recently conducted survey indicates that the incidence of TE in large medical centers in the United States has not changed since 1991. Thus, despite impressive progress made in many areas over the last 5 to 7 years, *Toxoplasma* remains an important priority for further basic and applied research.

It is now clear that *Toxoplasma* is clonally distributed. There are three predominant clones (I, II, and III) that have significant biological differences. Type II strains cause the majority of disease in humans.

Outstanding Research Needs

- There is little understanding of the role that parasite-versus-host genotypes play in disease, and it remains unclear why toxoplasmosis develops in only a subset of patients. Further research is needed to define the relationship of both the parasite and host genotypes to disease pathogenesis and clinical outcomes.

- Research is needed to carefully define the contribution clonal type may play in determining differences with respect to drug sensitivity, virulence, and immune response.

C. Pathogenesis

Important advances have been made in a number of areas related to the molecular biology, cell biology, immunology, and biochemistry of *T. gondii*. The availability of molecular genetic techniques for manipulation of *T. gondii* has dramatically increased over the last 3 to 4 years. Substantial progress has been made in understanding the cell biology of parasite attachment, invasion, and intracellular survival. There has been a convergence of findings on the host genetic and immunologic responses to infection. Important advances in biochemistry and metabolism have come in the identification and molecular characterization of parasite enzymes involved in nucleoside salvage. The need for fundamental research in the following areas remains great:

Outstanding Research Needs

- There is an urgent need to define fundamental metabolic pathways of the parasite, especially bradyzoites. Little is known about the basic metabolism of tachyzoites, and even less is known about the metabolic processes of bradyzoites. Intermediary metabolism studies are needed to define what pathways are present and how they differ from the mammalian host. Research in this area is needed and represents the best hope of ultimately identifying new targets and new therapies. As such, it deserves high priority.

- The host-parasite interaction is a critical area for future investigation.

    a. Research on the parasite cytoskeleton and on the mechanism of motility is warranted, given the involvement of the parasite cytoskeleton in host cell invasion.

    b. Characterizing the process of parasite secretion and defining the biological function of secreted proteins are essential for understanding the mechanism of intracellular infection. These studies will likely define new targets for therapeutic intervention.

    c. Research is necessary to increase understanding of the role that specific *T. gondii* genes play in virulence. Knowledge of these genes should provide the foundation necessary to develop new therapies and/or vaccines.

    d. Research is needed to identify and characterize genes involved in stage conversion from tachyzoite to bradyzoite or that are necessary for intracellular replication. The identification of essential gene functions will help define novel therapeutic targets.

    e. Although host genetic factors in murine and human systems are partially identified, there is a need to determine how chronic infection is established and how it may be modulated by the host immune system. The area that is the least clear, and one for which continued attention is needed, is definition of the combination of factors (parasite strain, host genetic background, immunologic components) that are involved in the reactivation of disease.

- Further development of stable episomal vectors, or those with inducible promotors, and of new strategies to disrupt essential genes or otherwise genetically manipulate these parasites is needed in order to better understand their biology. Research efforts should be devoted to

developing those tools. Construction of new libraries should also be supported to further define the genetic constituents of these pathogens and the genes they express at different stages of their life-cycles. The continued lack of an axenic culture system confounds mutant generation and recovery, and the NIH should support efforts to identify axenic culture conditions.

D. Diagnosis

Diagnosis of TE is usually made empirically, based on the presence of one or more characteristic lesions seen on head CT or MRI scans in a patient who is seropositive for *Toxoplasma*. A clinical response to empiric therapy is taken as presumptive proof of the diagnosis. *T. gondii* infection at other sites (such as the lungs) is diagnosed by identification of the organism in tissue or lavage specimens. Rapid diagnosis by PCR is now possible and is positive in up to three-fourths of blood and cerebrospinal fluid (CSF) from patients with TE. Nonetheless, this test is not widely available and, hence, is used in only a limited number of clinical settings.

Outstanding Research Needs

- Improved diagnostic tools are needed, but it is likely that there will be no simple solutions to the problem of distinguishing active from chronic infection. It is not obvious that PCR-based assays are the answer, and new approaches in this area are needed.

- Newly available monoclonal antibodies to parasite antigens as well as cloned genes expressed as recombinant full-length and/or fusion proteins could be used to develop assays and reagents for detection of specific parasite antigens in infected individuals.

E. Treatment and Prophylaxis

Standard therapy for toxoplasmosis consists of pyrimethamine and sulfadiazine. Clindamycin combined with pyrimethamine is an acceptable alternative therapy. A variety of additional agents, including azithromycin, clarithromycin, atovaquone, dapsone, doxycycline, and rifabutin show activity in animal models. Results from clinical trials with most of the above agents indicate that none can be effectively used as a single agent. One major limitation to all currently available agents is the lack of clinical efficacy against the latent form of the parasite, the bradyzoite, which is found within tissue cysts.

Bactrim appears to be effective as primary prophylaxis to prevent the emergence of TE in TE-seropositive AIDS patients. The combination of dapsone and pyrimethamine is also likely to be effective. As with primary or maintenance therapy, prophylaxis with a single agent (particularly pyrimethamine) is probably ineffective as primary prophylaxis.

Outstanding Research Needs

- High-throughput screens for microbicidal drugs should be applied to both tachyzoites and bradyzoites, using new systems developed for *in vitro* bradyzoite generation and for automated monitoring of parasite growth. These screens are probably best done by the pharmaceutical industry, with input from scientists in academia, although this may not be

realistic without more active encouragement of industry involvement. Since eradication of bradyzoites within tissues would eliminate the risk of recurrence from recrudescent disease, identification of drugs with this spectrum of activity is a particularly attractive goal. The development of conditions for *in vitro* differentiation of tachyzoites from bradyzoites, of stage-specific reagents, of transgenic parasites stably expressing enzyme markers (such as β-galactosidase), and of molecular genetic tools is likely to have a major impact on this priority. There also is no substitute for continued screening of promising compounds *in vivo*, since there are discrepancies between the utility of drugs *in vitro* and *in vivo* for *T. gondii*, at least in part due to differences in *in vitro* assay methodology.

- New drug targets should be identified through the definition of metabolic pathways, targeted sequencing efforts, further definition of the cell biology of invasion and intracellular infection, and new molecular approaches, including mutant generation.

- A human vaccine for *T. gondii* is neither practical nor appropriate. Vaccination of meat animals that represent an important source of human infection is, however, a viable approach because (1) such a vaccine can reasonably be tested for efficacy, (2) it would have its own market (*T. gondii* causes spontaneous abortion in sheep), (3) a great deal is known about how to formulate animal vaccines, and (4) such animals are kept under semicontrolled conditions. Although this research would not have immediate benefits, such an approach could ultimately massively reduce the number of chronically infected persons at risk for reactivation.

- The relative contribution to human infection of oocysts (in cat feces) versus tissue cysts (in undercooked meat) needs clearer definition. This information should be incorporated into proactive public health education programs that transmit the message that toxoplasmosis is a problem and infection with *Toxoplasma* is worth avoiding. Wide dissemination of simple recommendations to prevent acquisition of toxoplasmosis could ultimately have a wide impact in lowering the infected population at risk for reactivation.

VI. *Cryptosporidium* and the *Microsporidia* OIs

A. Introduction

Gastrointestinal OIs contribute significantly to morbidity and mortality rates observed in AIDS patients. Enteric infectious diseases annually compromise the health of millions of individuals worldwide. In fact, the single leading cause of early childhood mortality in many developing countries can directly be linked to diarrheal diseases. *Cryptosporidium parvum* has recently emerged as a leading cause of diarrheal illness in the immunologically naive and the immunocompromised, particularly AIDS patients. Severe illness in these individuals may predispose to life-threatening situations and is exacerbated by the absence of effective chemotherapy. The incidence of cryptosporidiosis in these patients varies widely depending on geographic and socioeconomic factors. In the United States, cryptosporidiosis has been reported as an AIDS-defining illness in ~3 percent of individuals. The true incidence, however, is more likely to approach 15 to 20 percent of U.S. AIDS patients as their situation worsens. In areas of the world such as Haiti and in portions of Africa, the incidence of infection with this parasite in

AIDS patients may approach 50 percent. Unfortunately, cryptosporidiosis will likely continue to be a major cause of diarrheal problems and will continue to be a cause of death in AIDS patients until appropriate prevention and treatment strategies are developed.

The awareness of *Cryptosporidium* as a major problem in the AIDS population has also resulted in increased appreciation of the presence of this parasite in the general population. Indeed, there is a growing concern over the spread of this organism through water due to several recent well-documented and highly publicized waterborne disease outbreaks in immunocompetent as well as immunocompromised populations in both the United States and in the United Kingdom. If *Cryptosporidium* is considered a potential threat to the general population in these developed countries, its impact as yet undefined in developing nations may be even more profound.

Along with *Cryptosporidium,* organisms of the phylum *Microspora* have increasingly been associated with enteric disease and other problems in AIDS patients. The microsporidia, as they are referred to collectively, actually represent several genera and species of parasites. Recent studies indicate that microsporidian infections may be as common in AIDS patients as are infections with *Cryptosporidium*. It is quite apparent from the published literature that far less is known about this group of organisms than is known about *Cryptosporidium*. Infections caused by these parasites are difficult to diagnose and, in virtually every instance, very little is known about their biology and host range. It also is not known if these parasites pose a threat to the general population. Furthermore, the microsporidia have proven difficult to treat.

It is very clear that basic and applied research efforts must continue to be directed toward studying these opportunistic enteric parasites of humans. The consensus opinion of investigators working on these organisms is that improvements to understanding the basic biology, biochemistry, development, molecular biology, epidemiology, and host-parasite relationship of these organisms is essential to making advancements that will lead to developing strategies aimed at preventing and controlling infections caused by them. It is equally apparent that what has been learned about these organisms has been, and must continue to be, fostered through NIH-sponsored research and communication networks.

B. Epidemiology/Relevance to AIDS

Infections with *Cryptosporidium* and the *Microsporidia* are not reportable illnesses in the vast majority of the United States. Indeed, these organisms are not part of a standard ova and parasite examination. Because of this and because many technicians are poorly trained in making these diagnoses, the true incidence and prevalence of these infections remain unknown in both the AIDS and general population. Recent data suggest, in fact, that the incidence of both may be increasing in the AIDS-affected population. The specific recommendations given below must be intimately linked with those presented under the diagnosis heading (Section D below).

Outstanding Research Needs

- Epidemiologic studies are needed to determine the true incidence of these infections in HIV-infected persons and the threat that they represent to the general population. Basic questions of host range and specificity along with transmission routes need to be determined for the *Microsporidia*.

- Determine whether isolate/strain variation for *Cryptosporidium* exists and if this impacts on pathogenic potential. Develop isolate-specific PCR probes to distinguish isolated strains.

C. Pathogenesis

Mechanisms by which both *Cryptosporidium* and the *Microsporidia* produce pathogenesis remain largely unknown. In large part this is due to the lack of appropriate *in vitro* and *in vivo* models to study these infections. Evidence suggests that *Cryptosporidium* may produce a toxin, but this has not been substantiated. Furthermore, many of the *Microsporidia* have the potential to disseminate within infected individuals, thus enhancing their pathogenic potential. In addition, it is not known how the course of infection and disease may be influenced by host age, nutritional status, immune status, and parasite isolate differences.

Outstanding Research Needs

- Develop and improve both *in vitro* and *in vivo* models that can be used to address issues of basic organism biology and pathophysiology, immune responsiveness, prophylaxis and therapy, and differential parasite life cycle stage production. This remains the highest priority of research in this area.

- Determine molecular and pathophysiologic mechanisms of disease and explore potential treatments directed at amelioration of deranged physiology.

- Gain a more fundamental knowledge of the host-parasite relationship during *Cryptosporidium* infection and better characterize immune responsiveness in both immunocompetent and immunocompromised hosts. In addition, determine if latent infections occur.

D. Diagnosis

Diagnosis of both *Cryptosporidium* and the *Microsporidia* depends on physician awareness of these infections and subsequent specific recommendation that they be tested for in the clinical diagnostic laboratory. At present, such testing is laborious, expensive, and often performed by poorly trained technicians.

Outstanding Research Needs

- Diagnostic methods are desperately needed for the *Microsporidia* and more economical tests are needed for *Cryptosporidium.*

- Enhanced detection methods to determine viability of the *Cryptosporidium* oocyst in water are needed in order to evaluate new paradigms of water treatment.

E. Treatment and Prophylaxis

At present no treatment other than supportive rehydration therapy exists for *Cryptosporidium*, and only albendazole has proven somewhat efficacious for treating certain *Microsporidia* infections. Prophylactic treatments are lacking for both.

Outstanding Research Needs

- Identify molecular and biochemical targets for chemotherapeutic and immunotherapeutic approaches to controlling infection.

- Give strong consideration to the development of *Cryptosporidium* vaccination strategies that might enhance mucosal responsiveness in humans or control infections in domestic livestock, which could serve as a source of infection to humans.

- Support development of centralized facilities for the production, purification, distribution, and potential cryopreservation of parasites. To some extent, this may impact the desirability for a standardization of *in vitro* and *in vivo* model systems.

- Provide realistic funding support in terms of amount and time to conduct the above research and commit to bring bright, young, energetic investigators into this arena of research.

VII. Opportunistic Infections in Pediatric Patients

A. Introduction

As heterosexual transmission of HIV increases, pediatric patients constitute an ever-enlarging subset of HIV-infected patients, both in the United States and around the world. Improved antiretroviral therapy has expanded the life span of pediatric patients; many are now living to adolescence. While considerable advances have occurred with regard to recognition of the manifestations of the disease, the prevention of vertical transmission, and pediatric prophylaxis and treatment, our understanding of the predisposing factors and the pathogenesis of OI in pediatric patients is more rudimentary. The spectrum of OI in pediatric patients, the fact that these infections often develop within the context of a developing immune system with no prior exposure to the relevant pathogens, and the chance for early intervention distinguish these children from adult patients and make studies of OI in pediatric patients a compelling scientific issue.

The study of OI in HIV-infected children differs in two major aspects from ongoing investigations in adult patients.

B. Epidemiologic Distinctions

1. Acquisition as a consequence of vertical transmission from heterosexually infected females

2. Different spectrum of infections: e.g., recurrent infections with encapsulated bacteria; frequent involvement of proliferative viral disease (LIP); relative paucity of toxoplasmosis or Kaposi's sarcoma

3. Development of disease in the naive, nonimmune host

4. Opportunities for prophylaxis or early intervention.

C. Microbiologic Distinctions

1. Primary infection with *Pneumocystis carinii,* the most common AIDS indicator infection in HIV-infected pediatric patients

2. Lymphoid interstitial pneumonitis due to Epstein Barr virus, the second most common AIDS indicator condition

3. Recurrent pneumococcal bacteremia, the third most common infection

4. Mucosal candidiasis, the fourth most common condition and one that significantly impedes nutrition and growth.

Outstanding Research Need

- A specific focus on pediatric OIs is required, since there are both epidemiologic and microbiologic features that distinguish pediatric infections from those manifest in adulthood.

- Lengthened survival due to improvements in antiretroviral therapy and adjuvant measures should facilitate the study of acquisition, natural history, pathogenesis, and modes of intervention in OIs that are unique to pediatric HIV infection.

- Studies must be performed in children to better understand and treat infectious complications in this unique group of patients with AIDS.

VIII. Emerging/Unrecognized Pathogens

A. Introduction

New laboratory approaches have revealed previously unknown opportunistic microbial pathogens in persons with HIV infection. These approaches have included improved culture-based methods as well as molecular, culture-independent methods based on consensus amplification of phylogenetically useful sequences and on subtractive methods. Some of these organisms are implicated in the causation of well-known as well as more recently described clinical syndromes. Examples of recently characterized microbial pathogens in HIV-infected persons include *Bartonella henselae,* Kaposi's sarcoma-associated herpesvirus (KSHV), *Mycobacterium genavense* and other poorly characterized or uncultivated mycobacteria, and *Cyclospora*. These discoveries have led to new diagnostic assays, have suggested new potential therapies and prophylactic strategies, and have expanded our understanding of microbial diversity. They also emphasize our ignorance of the true diversity of pathogenic microorganisms that play significant roles in the clinical outcome of persons with HIV infection.

Despite these recent conceptual and technical advances, little attention has been focused upon the further characterization of these recently identified, or as yet unidentified, cultivation-resistant opportunistic pathogens. Current funding priorities are heavily biased toward a relatively small number of opportunistic pathogens that are readily detectable. Organisms that are cultivatable *in vitro* are heavily favored over those that are not (e.g., *M. avium* versus *M. genavense*). To the Subpanel's knowledge, no NIH funds are being used to support broad-based molecular searches for novel or unrecognized opportunistic microbial pathogens in HIV-infected persons. If one accepts the possibility that unidentified microorganisms play significant roles in such clinical syndromes as chronic diarrhea and chronic fever, then it is clear that current funding priorities completely ignore these areas of potential gain. Furthermore, few NIH funds are now being devoted to further the understanding of newly discovered microbial pathogens such as KSHV and *B. henselae* (regarding pathogenesis, epidemiology, clinical correlations, diagnosis, and therapy). The sequence-based identification of an uncultivated pathogen may lead to serologic or culture-based detection methods, the subsequent discovery of previously unsuspected roles in other diseases, and/or novel pathogenic mechanisms. Examples include the discoveries of *B. henselae* as the dominant etiologic agent of cat scratch disease, *B. quintan* seroreactivity in 20 percent of an indigent population in Seattle, and comparable rates of bacteremia for *M. avium* complex and *M. genavense* in HIV-infected persons. The following recommendations address the need for such broad-based searches in HIV-infected persons as well as research priorities concerning several recently described opportunistic pathogens.

B. Epidemiology and Pathogen Discovery

Certain clinical syndromes in HIV-infected persons may suggest the presence of currently unrecognized microbial pathogens; examples include chronic unexplained diarrhea, persistent or recurrent unexplained fevers with negative routine blood cultures, non-Hodgkin's lymphomas, and clinical subsets of the AIDS dementia complex (ADC)(>10 percent of HIV-infected persons) and AIDS wasting syndrome (>25 percent of HIV-infected persons). These clinical syndromes represent important settings for broad-based molecular searches for potential microbial pathogens. Recently described but poorly characterized pathogens, such as the Whipple's disease bacillus, may be associated with nonclassical disease in HIV-infected persons. Ignored or unrecognized opportunistic microorganisms may act as cofactors with well-known organisms to cause as yet unexplained AIDS-related disease manifestations. All of these syndromes are likely to become more common among HIV-infected persons as antiretroviral therapy and prophylactic antimicrobials prolong survival in this population. In addition, increasing use of prophylactic antimicrobials in HIV-infected persons will select for the emergence of new, intrinsically resistant opportunistic pathogens, as well as drug-resistant variants of previously characterized microorganisms.

We may anticipate the emergence of important pathogens in HIV-infected persons by turning to several nontraditional settings. Emerging pathogens in animals or endosymbionts in animals and insects may be particular threats to immunocompromised human hosts. Other opportunistic pathogens originate in the host commensal microbial flora; these flora may be an important target for study in diverse geographic locations. The "emergence" of *Penicillium marneffei* in U.S. HIV-infected persons following its importation from southeast Asia speaks to the importance of global surveillance.

Outstanding Research Needs

- The NIH needs to support broad-based innovative molecular and culture-based investigations to identify previously unrecognized opportunistic pathogens in HIV-infected persons. Certain unexplained clinical syndromes, such as those mentioned above, should be targeted. Particularly promising experimental approaches include consensus PCR amplification of conserved, phylogenetically useful microbial sequences (e.g., ribosomal DNA), and representational difference analysis (RDA). Careful selection of clinical samples and appropriate controls is critical (see the next recommendation). In the absence of proof of Koch's postulates, special attention should be placed upon data that support a causal association between molecular information and pathology. The NIH must recognize and accept the relatively high-risk nature of such investigations. Two-year, $150,000 to $200,000 pilot-feasibility grants may be a useful funding approach.

- There is a need for the NIH to establish a clinical specimen bank (body fluids and tissues) from HIV-infected persons with designated syndromes of unknown but potentially infectious etiology. Specimens containing suspected pathogens, or isolates that resist identification should also be collected. The specimen bank(s) should include matched specimens as controls. The bank(s) would serve as repositories of samples for which qualified investigators could apply for access. Carefully procured and catalogued specimens of this type are difficult to secure and, as a widely available valuable resource, would encourage more numerous and productive investigations of new putative pathogens. The ACTG network might offer an attractive source of samples. For example, bronchoscopic, endoscopic, liver, lymph node, and whole blood samples might be identified, catalogued, and saved during the course of multicenter protocols. One of the advantages of a well-established network such as the ACTG is that critical clinical information and followup data would be available for correlation with molecular data. Actual, prospective "real-time" specimen collection will be necessary to track sporadic or unanticipated clinical syndromes; donor identification alone might be sufficient with later specimen collection for unexplained but common clinical syndromes.

- The NIH should collaborate with and lend support to the CDC's Emerging Infections program. This program currently comprises four regional centers (in Connecticut, Minnesota, Oregon, and California) devoted to epidemiologic surveillance and analysis of unexplained critical illnesses and deaths in HIV-negative hosts. A similar network should be established for epidemiologic surveillance and laboratory investigation of unexplained critical illnesses and deaths in HIV-infected persons. The NIH should encourage this type of program for HIV-infected persons from regions of the world with high HIV endemicity.

- There is a need to address the following issues regarding specific pathogens: KSHV—risk factors for infection and disease, reservoir, virus population structure, origins of the virus, mechanisms of transmission, incubation period, host immune response to infection and disease, and role in other diseases; B. henselae—mechanisms of transmission, geographic and temporal fluctuations in disease prevalence, and population structure; *Cyclospora*—relative importance as cause of chronic diarrhea in HIV-infected persons.

C. Pathogenesis

For those microorganisms recently identified, very little is known concerning pathogenic mechanisms. The investigation of these mechanisms and subsequent host responses may elucidate fundamental biological processes and reveal important concepts concerning HIV pathogenesis and HIV-associated host immunologic defects. For example, what is the nature of the HIV-induced host defect that permits KSHV and *B. henselae* to induce an angiogenic host response preferentially in HIV-infected persons, or EBV and KSHV to induce oncogenic transformation in these hosts? What effect does HIV infection have on the composition of the host commensal microflora and, hence, on the host response to mucosal or cutaneous antigenic stimulation? Insights into pathogenic mechanisms also often lead to improved therapeutic and prophylactic approaches.

Recommendations

- **The NIH should fund basic laboratory investigations of pathogenic mechanisms used by recently identified opportunistic pathogens in HIV-infected persons.**

- **Interactions between HIV and recently identified opportunistic pathogens should be explored. In addition, the contributions of one opportunistic microbial agent to the pathogenicity of another agent may be an important area for study.**

- **The impact of HIV infection on the composition of the host commensal microbial flora should be evaluated as an important source of emerging pathogens.**

- **The following issues should be addressed regarding specific pathogens: KSHV and *B. henselae*—association of specific clones with angioproliferation or transformation, mechanisms of pathogen-associated angiogenesis or oncogenic transformation, mechanisms of cellular and tissue tropism, development of animal models, role of host cytokines and immune responses in causation of disease and host pathology, ability of a pathogen to modulate host responses; KSHV—development of methods for cultivation, assessment of latency; *B. henselae*—differential host tissue response in immunocompromised versus immunocompetent hosts.**

D. Diagnosis and Detection

These recently identified or currently unidentified microorganisms are, by definition, fastidious or resistant to cultivation and poorly characterized, so that traditional diagnostic methods are inadequate or suboptimal for their detection and for diagnosis of disease. Until improved methods are available, only the most rudimentary understanding of these infections will prevail. Two recent approaches for the identification of novel organisms are (1) consensus PCR amplification of conserved, phylogenetically useful microbial sequences and (2) Representative Difference Analysis (RDA). The first approach may be useful for identifying members of the bacterial domain, the fungi, and other selected monophyletic groups. Novel viruses can be identified if prior information is available from which to target a selected group of agents. RDA may prove most useful in the detection of occult viruses. Neither approach is currently applicable to routine diagnosis.

Outstanding Research Need

- The NIH should support efforts to develop novel and rapid methodologies for the detection and identification of opportunistic pathogens in HIV-infected persons.

E. Treatment and Prophylaxis

Obviously, treatment and prophylaxis are relevant only to currently identified opportunistic pathogens. Those that have been recently identified are important causes of morbidity and mortality in many cases. For the reasons outlined above, little information is available concerning appropriate treatment and prophylactic strategies for these organisms.

Outstanding Research Need

- Regarding specific pathogens, the following issues should be addressed:
  KSHV—drug susceptibility, efficacy of foscarnet as therapy for KSHV infection;
  B. henselae—antibiotic efficacy in cat scratch disease versus bacillary angiomatosis, appropriate duration of therapy.

Appendix

Biographies of Panel Members

Marilyn S. Bartlett, M.S., is Professor, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, where she directs the clinical Mycology and Parasitology laboratory and the *Pneumocystis carinii* research laboratory. She produced a short-term culture method for *P. carinii* that has allowed identification of effective compounds, including 8-aminoquinolines, for treatment of *P. carinii* pneumonia; developed inoculated rat and mouse animal models for use in *P. carinii* research; and initiated epidemiologic and transmission studies using molecular biology techniques established by Dr. Lee at Indiana University with human samples collected in her laboratory. Ms. Bartlett was the editor of the parasitology section in American Public Health's 7th Edition of *Diagnostic Procedures for Mycotic and Parasitic Infections* and contributed to the American Society for Microbiology's *Manual of Clinical Microbiology* and *Clinical Microbiology Procedures Handbook.* She is an author of 85 refereed journal publications.

Barry R. Bloom, Ph.D., is an Investigator at the Howard Hughes Medical Institute and the Weinstock Professor of Microbiology and Immunology at the Albert Einstein College of Medicine in New York. He received his B.A. degree and an honorary Sc.D. from Amherst College and his Ph.D. from the Rockefeller University. Dr. Bloom chaired the Tuberculosis Committee at the World Health Organization (WHO) and is currently the Chair of the Scientific and Technical Advisory Committee to the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Dr. Bloom served as a consultant to the White House on International Health Policy from 1977-78. He has served as a member of the National Advisory Council of NIAID, its AIDS subcommittee, and the U.S. National Vaccine Advisory Committee. He is currently Co-Chair of the Board on International Health of the National Research Council of the National Academy of Sciences (NAS). He serves as a member of the WHO Ad Hoc Committee for Research Priorities and on the NAS Committee on Criteria for Federal Support of Federal Research and Development. In 1991, he received the first Bristol-Myers Squibb Award for Distinguished Research in Infectious Diseases. Dr. Bloom also is a member of the NAS, a member and Councillor of the Institute of Medicine, and a member of the American Academy of Arts and Sciences.

Yuan Chang, M.D., has been Assistant Professor in the Department of Pathology at Columbia University College of Physicians and Surgeons since 1993, after finishing her pathology fellowship training at Stanford University Medical Center. She has been engaged in the virologic and epidemiologic characterization of the Kaposi's sarcoma-associated herpesvirus (KSHV), a new human herpesvirus identified and sequenced in her laboratory. She is the recipient of a James S. McDonnel Scholar Award for cancer research.

Donald M. Coen, Ph.D., has been Professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School since 1992, where he previously was Assistant Professor of Pharmacology since 1982. Dr. Coen has extensive research experience in efforts to discover new antiviral drugs, studies of antiviral drug mechanisms and resistance molecular analyses of viral enzymes and gene expression, and studies in the area of viral pathogenesis.

William L. Current, Ph.D., received his doctorate in Life Sciences from the University of Nebraska in 1977 and was on the faculty of Auburn University for 7 years before joining Lilly Research laboratories in 1984. During the past 5 years as a Senior Research Scientist in the Infectious Disease Research Unit of Lilly Research Laboratories, he has supervised and participated in basic research aimed at the discovery of drugs to treat life-threatening infections in the immunocompromised host caused by opportunistic eukaryotic pathogens, including *Cryptosporidium, Toxoplasma, Pneumocystis, Candida, Aspergillus,* and *Cryptococcus.* Dr. Current has coauthored more than 100 papers and book chapters, was a member of several editorial boards as well as advisory committees evaluating research programs, and served as a member of an NIH study section.

Melanie T. Cushion, Ph.D., is presently Associate Professor of Medicine, Department of Internal Medicine, University of Cincinnati College of Medicine, where she had previously been Assistant Professor of Medicine since 1994. A member of the American Society for Microbiology, she has received numerous awards, including the Academic and Tuition Scholarship at St. Thomas Institute and the Academic Trustee Scholarship at St. Joseph's College. She is the author of many publications, including one on the effects of pentamidine and other inhibitors on *Pneumocystis carinii* by ATP content and pulsed field gel electrophoresis. Dr. Cushion is a member of the Graduate Program Committee and the Pathobiology and Molecular Medicine Committees at the University of Cincinnati College of Medicine; previously, she was a member of the Graduate Thesis Committee for the Department of Molecular Genetics, Biochemistry, and Microbiology.

George Deepe, Jr., M.D., received his M.D. from the University of California School of Medicine and completed his residency in Internal Medicine at Southern Illinois University School of Medicine. He completed his training in Infectious Diseases at the University of Kentucky and is Professor and Chief of the Division of Infectious Diseases at the University of Cincinnati College of Medicine. Dr. Deepe's research interests are in the identification of antigens from *Histoplasma capsulatum* that can be used as a vaccine. He also is investigating the molecular and cellular determinants of protection.

John E. Edwards, Jr., M.D., is Professor of Medicine at the University of California at Los Angeles School of Medicine. He previously was a Guest Worker at the Laboratory of Clinical Investigation, NIAID, NIH, from 1984-85. Dr. Edwards received his B.A. degree in Zoology at Pomona College in Claremont, CA, and his M.D. degree at the University of California, Irvine College of Medicine. He has received numerous honors and awards, including the Infectious Disease Society of America Meritorious Abstract Award in 1993 and the George Gee Jackson Visiting Professorship of the Infectious Diseases Society of America in 1989.

Jerrold Ellner, M.D., received his A.B. from Cornell University in 1966 and his M.D. from the Johns Hopkins University in 1970. He completed his residency in medicine at the Johns Hopkins Hospital and his postdoctoral training in Infectious Diseases and Immunology at the Laboratory of Clinical Investigation, NIAID, NIH. Dr. Ellner joined the faculty at Case Western Reserve University in 1976, where he was appointed Chief of the Division of Infectious Diseases at University Hospitals in 1979 and is currently Professor of Medicine and Pathology. He is Chair of the Tuberculosis Panel of the U.S.-Japan Cooperative Medical Sciences Program and a member of the National Advisory Council for Allergy and Infectious Diseases; the Immunology

of Mycobacterial Diseases Steering Committee, WHO; and the HIVNET Steering Committee, NIH. He formerly was a member and Chair of the Bacteriology and Mycology-1 study section at the NIH and a member of the Public Health Service Advisory Council for Elimination of Tuberculosis. Dr. Ellner received the Squibb Award from the Infectious Diseases Society of America in 1990 and is a member of the Association of American Physicians. His major research interest is the immunopathogenesis of tuberculosis and interactions of tuberculosis with HIV-1 infection.

Stanley Falkow, Ph.D., has been Professor of Microbiology and Immunology and Medicine at Stanford University School of Medicine since 1985. He received his B.S. degree from the University of Maine and his Ph.D. from Brown University. Upon completion of his graduate studies, Dr. Falkow became a staff member at the Walter Reed Army Institute of Research in the Department of Bacterial Immunology and was later named the Assistant Chief of the Department. He joined the faculty of Georgetown University Medical School as Professor of Microbiology in 1966 and later joined the faculty of the Department of Microbiology and Immunology at the University of Washington Medical School. From 1981-85, he served as Chairman of the Department of Medical Microbiology at Stanford University School of Medicine. Dr. Falkow has made significant contributions to the field of microbiology and is recognized worldwide for his observations related to molecular mechanism of bacterial pathogenesis. Among his honors and awards are election as a member of the National Academy of Sciences (1986), the Altemeier Medal from the Surgical Infectious Diseases Society of America (1990), the Squibb Award from the Infectious Diseases Society of America (1979), the Howard Taylor Ricketts Award (1995), the Paul Ehrlich-Ludwig Darmstaedter Prize (1981), and honorary doctorates in Europe and the United States. Dr. Falkow has served as an editorial board member of many prestigious journals and belongs to numerous professional organizations. One of Dr. Falkow's greatest accomplishments has been that of serving as mentor to many individuals who have continued their successes in the study of microbial pathogenesis.

Gerald R. Fink, Ph.D., is Director of the Whitehead Institute for Biomedical Research in Cambridge, MA, and American Cancer Society Professor of Genetics at the Massachusetts Institute of Technology. Previously, Dr. Fink was Professor of Genetics from 1976-79 and professor of Biochemistry from 1979-1982 at Cornell University. He is a member of the Board of Trustees of Cold Spring Harbor Laboratory, a Non-Resident Fellow of the Salk Institute, a member of the Scientific Advisory Boards of the Biozentrum in Basel and the Howard Hughes Medical Institute, and a member of the Board of Scientific Counselors of the National Institute of Child Health and Human Development, NIH. He served as the President of the Genetics Society of America from 1988-89. Dr. Fink serves on the editorial boards of *Genes and Development, Molecular Biology of the Cell*, and *Trends in Genetics*. He was elected to membership in the National Academy of Sciences in 1981. Other honors include the Wilbur Lucius Cross Medal from the Yale Graduate School Association, the Emil Christian Hansen Foundation Award for Microbiological Research, the Yale Science and Engineering Award, the Genetics Society of America Medal, an Honorary Doctor of Science from Amherst University, the National Academy of Sciences-U.S. Steel Prize in Molecular Biology, and a Guggenheim Fellowship. By combining traditional genetics and modern molecular biology, Dr. Fink has made major strides in understanding cell growth and metabolism at the molecular level.

Harold S. Ginsberg, M.D., is an Expert Scientist at NIAID and the Eugene Higgins Professor of Medicine and Microbiology, Emeritus, College of Physicians and Surgeons at Columbia University. He is a member of the National Academy of Sciences and the Institute of Medicine. He serves on the Board of Governors of the American Academy of Microbiology and the U.S. National Committee for the International Union of Microbiological Societies. He cochaired the Institute of Medicine Roundtable for the Development of Drugs and Vaccines Against AIDS and has chaired the NIH Ad Hoc AIDS Study Section. He also has been a Vice President, International Committee for Nomenclature of Viruses of the International Association of Microbiological Societies. Dr. Ginsberg has received numerous awards, including the Senior U.S. Scientist, Humboldt Award; the Physicians and Surgeons Distinguished Service Award; and the Bristol-Myers Squibb Award for Distinguished Achievement in Infectious Disease Research. He is an Honorary Fellow of the American Association for the Advancement of Science and was a Fogarty International Scholar in 1992. After graduating from the Tulane University School of Medicine, Dr. Ginsberg served in World War II, achieving the rank of Lieutenant Colonel, and was awarded the Legion of Merit.

Carlton Hogan is Training Coordinator at the Statistical Center for the Community Programs for Clinical Research on AIDS (CPCRA). He is also the editor of *PWAlive*, a journal by, for, and about persons living with AIDS. Previous to his tenure at the CPCRA, he was at the Minnesota AIDS Project, first as designer of the IDU outreach program and then as Health Education Case Manager. Mr. Hogan attended the White House Conference on AIDS and has sat as an invited guest on the FDA Antiviral Advisory Council.

James M. Hogle, Ph.D., has been the Edward S. Harkness Professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School since July 1991. He has served as Chair of the Committee on Higher Degrees in Biophysics, which administers the interdepartmental Ph.D. program in Biophysics at Harvard University and Harvard Medical School, since July 1992. Dr. Hogle received his B.S. in Biochemistry from the University of Minnesota at Minneapolis in 1972 and his Ph.D. in Biochemistry from the University of Wisconsin at Madison in 1978. He did his postdoctoral training at Harvard University from 1978-1981 and subsequently joined the Department of Biochemistry as a Research Associate from 1981-82. From 1982-85, Dr. Hogle was an Assistant Member in the Department of Molecular Biology at The Research Institute of Scripps Clinic, where he was an Associate Member from 1985-86 and a Member from 1987-1991. He received the American Association for the Advancement of Science Newcomb-Cleveland Prize in 1985 and the Wallace P. Rowe Award for Excellence in Virology in 1991. Dr. Hogle has authored or coauthored over 50 scientific publications, and he serves on the editorial boards of *Journal of Virology*, *Virus Genes*, and *Protein Science*. His research focuses on the structure and function of viruses and viral proteins, using a combination of x-ray crystallographic and molecular biological approaches. His current research involves the study of several viruses including poliovirus, herpesviruses (HSV and CMV), and hepatitis virus.

Margaret K. Hostetter, M.D., holds the American Legion and Women's Auxiliary Heart Research Chair in Pediatrics at the University of Minnesota, where she is Professor of Pediatrics. She has won recent research awards from the Society for Pediatric Research, the American Academy of Pediatrics, and the Samuel Rosenthal Foundation. Her studies involve the pathogenesis of bacterial and fungal infections.

William R. Jacobs, Jr., Ph.D., is presently Associate Professor in the Department of Microbiology and Immunology and the Department of Molecular Genetics, and Associate Investigator at the Howard Hughes Medical Institute. From 1990-92, he was Assistant Professor in the Department of Molecular Genetics at the Albert Einstein College of Medicine. Dr. Jacobs is the author of many publications, including one that compared an integrated map of the genome of the tubercle bacillus *Mycobacterium tuberculosis* H37Rv with *M. leprae*.

Keith Joiner, M.D., is Professor of Medicine, Cell Biology, and Epidemiology at Yale University School of Medicine, where he has been since September 1989. He had previously been a senior investigator at the NIH from 1980-89, where he was Head of the Unit on Microbial Pathogenesis within the Laboratory of Parasitic Diseases, NIAID, NIH, from 1987-1989. Dr. Joiner's research area is microbial pathogenesis. His initial research was on the interaction of the complement cascade with pathogenic bacteria and protozoan parasites. His work now focuses on the cell biology of intracellular parasitism with *Toxoplasma gondii*.

Earl R. Kern, Ph.D., completed his doctorate in the Department of Microbiology at the University of Utah in 1973. He was a member of the faculty of the University of Utah School of Medicine from 1973-1988 and attained the rank of Professor in 1985. In 1988, he joined the University of Alabama at Birmingham, where he is Research Professor in the Departments of Pediatrics, Microbiology, and Comparative Medicine. He is also a Senior Scientist at UAB's Center for AIDS Research and the Comprehensive Cancer Center. He has made a major contribution to the development of experimental viral infections in animal models for use in testing the efficacy of new antiviral compounds. Dr. Kern is a senior author or coauthor of more than 100 publications and has presented or coauthored approximately 175 presentations at national or international meetings. He has had numerous invitations to present seminars at pharmaceutical companies and universities and review lectures at national and international meetings in the areas of animal models, herpesviruses, and antivirals. Dr. Kern has served as a consultant in the area of antiviral chemotherapy and is an expert in preclinical evaluation of antiviral drugs. He is the author of the chapter "Preclinical Evaluation of Antiviral Diseases of Man." He is currently President-Elect of the International Society for Antiviral Research and an Editor-in-Chief of *Antiviral Research*. Dr. Kern is a member of the editorial boards of a number of other scientific journals involving antiviral chemotherapy.

Richard Locksley, M.D., is the Chief of the Division of Infectious Diseases and Professor of Medicine, Microbiology, and Immunology at the University of California, San Francisco. He recently served as the Chair of the Tropical Medicine and Parasitology study section for the NIH and is currently the Chair of the Pathogenesis Committee for the World Health Organization. He serves on the editorial boards for *Immunity*, the *Journal of Experimental Medicine*, and the *Journal of Clinical Investigation*. Dr. Locksley is recipient of a Molecular Parasitology Scholar Award from the Burroughs Wellcome Fund and the Bailey K. Ashford Medal from the American Society of Tropical Medicine and Hygiene. He has extensive research interest in the development and function of effector T cells, cytokine biology, and the genetics of susceptibility to infectious and parasitic diseases.

George Miller, M.D., is a world leader in elucidating the biology of Epstein-Barr virus (EBV), a human oncogenic virus. His contributions are basic to understanding latent viral infection, regulation of gene expression, and viral oncogenicity. He has been at Yale University since 1969

and is presently the John F. Enders Professor of Pediatric Infectious Diseases and Professor of Epidemiology and Molecular Biophysics and Biochemistry. Dr. Miller is currently serving on the Burroughs Wellcome Fund Career Awards Advisory Committee and the Damon Runyon Scholar Award Committee and is on the Board of Scientific Advisors of St. Jude's Research Hospital. He is a member of numerous professional societies, including the American Society for Clinical Investigation, the Association of American Physicians, the Infectious Diseases Society of America, the American Society for Virology, and the American Society for Microbiology, where he was past DNA Virus Chairman. Dr. Miller is the recipient of several awards, including the Squibb Award, Enders Award, Macy Faculty Scholar Award, and the American Cancer Society Scholar Award. He is presently on the editorial board of *Pediatric Research* and formerly served on the editorial boards of the *Journal of Virology*, *Virology*, and the *Journal of Infectious Diseases*, among others.

Edward Mocarski, Ph.D., is Professor and Chair of the Department of Microbiology and Immunology at Stanford University School of Medicine, where he began as an Assistant Professor in 1983. He received his Ph.D. training at the University of Iowa with Dr. Mark Stinski, where he began his work on cytomegalovirus (CMV) biology, and pursued postdoctoral training with Dr. Bernard Roizman at The University of Chicago, where he studied herpes simplex virus replication. Dr. Mocarski's expertise is in the molecular biology and pathogenesis of human herpesviruses, with particular attention to human CMV, a key pathogen in AIDS patients as well as other immunocompromised individuals. His group has investigated the key functions necessary to regulate viral gene expression and to replicate the viral genome and has described genes that influence tropism for particular tissues in the infected host. Recent efforts have focused on defining the genes expressed during latency in progenitors of the granulocyte-macrophage lineage and on studying the mechanism of reactivation. He is currently training 4 graduate students and 6 postdoctoral fellows and has trained 7 Ph.D. students and 18 postdoctoral scientists over the past 13 years.

John Perfect, M.D., is Associate Professor of Medicine and Assistant Professor of Microbiology at Duke University Medical Center, Durham, NC. His research efforts include studies on molecular pathogenesis in *Cryptococcus neoformans*, molecular target identification for antifungal therapy, and therapeutic evaluation of antifungal drugs in preclinical trials. He is a member of the NIAID-sponsored Mycoses Study Group, which conducts collaborative studies on clinical drug trials and epidemiology for fungal infections. He is an Infectious Disease Consultant at Duke University Medical Center.

David A. Relman, M.D., is Assistant Professor of Medicine (Infectious Diseases and Geographic Medicine) and of Microbiology and Immunology at Stanford University School of Medicine and a staff physician at the Veterans Affairs Palo Alto Health Care System. His research interests concern the molecular basis of bacterial pathogenesis and the development of molecular methods for the identification of novel, uncultivated microbial pathogens. Dr. Relman has identified the causative agents of bacillary angiomatosis and Whipple's disease, among other diseases. He has been a consultant to the U.S. Congress Office of Technology Assessment on genotype-based bacterial identification and a collaborator with the U.S. Centers for Disease Control and Prevention's Emerging Infections Program. Dr. Relman received the Biomedical Scholar Award from the Lucille P. Markey Charitable Trust and the Baxter MicroScan Diagnostics Young Investigator Award from the American Society for Microbiology.

Larry R. Stanberry, M.D., Ph.D., received his degrees in Pharmacology from the University of Illinois in Chicago. He completed a residency in Pediatrics at the University of Texas Southwestern Medical School in Dallas and fellowship training in Infectious Diseases at the University of Utah in Salt Lake City. In 1982, he joined the faculty of the University of Cincinnati College of Medicine, where he is currently the Pauline and Lawson Reed Professor of Pediatrics and Director of the Division of Infectious Diseases at the Children's Hospital Research Foundation. His research interests include the pathogenesis and control of genital herpes simplex virus infections, the basic biology of viral latency, and the development and preclinical evaluation of intravaginal chemoprophylactic agents designed to prevent sexually transmitted diseases as well as of cytomegalovirus and herpes simplex virus vaccines. He is currently the principal investigator of a multinational, multicenter, industry-sponsored trial of a subunit herpes simplex virus vaccine for the prevention of genital herpes.

Charles Sterling, Ph.D., is Professor and Head of the Department of Veterinary Science and Director of the Undergraduate Program in Microbiology at the University of Arizona. He previously served as Associate Director of the University's Biotechnology Program. He recently chaired the NIH AIDS-Related Research study section dealing with opportunistic infections. He has extensive research experience with cryptosporidiosis in immunologically naive and immunocompromised populations, *Cyclospora* infections in humans, monoclonal antibodies in diagnostic parasitology and immunotherapy, and the epidemiology and immunology of enteric protozoan infections.

Richard R. Tidwell, Ph.D., is Director of the Division of Cellular Biology and Biological Chemistry and Professor of Pathology and Medicinal Chemistry at the University of North Carolina at Chapel Hill. He is a consultant to numerous pharmaceutical companies and serves as the Interim President of CaroTech, Inc., Durham, NC. Dr. Tidwell received his Ph.D. in Medicinal Chemistry in 1974 from the University of Tennessee Center for Health Services in Memphis. He has coauthored more than 70 manuscripts in peer-reviewed journals and 5 book chapters. He has been issued 8 patents and has filed an additional 13 patent applications. Dr. Tidwell's current work is directed toward the design, synthesis, and preclinical testing of new agents for the treatment of AIDS-associated opportunistic infections. He is the principal investigator of a National Cooperative Drug Development Group (NCDDG) grant to develop new drugs to treat opportunistic infections. This group includes projects at Duke University, Georgia State University, and Auburn University as well as the University of North Carolina at Chapel Hill. One of the compounds developed in his laboratories is due to begin clinical trials this spring for the treatment of *P. carinii* pneumonia. Several other drugs are in preclinical development for the treatment of *C. parvum, C. neoformans, C. albicans,* and *M. tuberculosis* infections.